

## WHAT IS CLAIMED IS:

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1. A method for identifying a compound that is an agonist of intracellular signaling effected by GPI-anchored receptors in nervous system cells comprising (i) incubating said nervous system cells having GPI-anchored receptors with a test compound and (ii) determining whether intracellular signaling has been effected in said cells.
2. The method of Claim 1, wherein said nervous system cells express GFR $\alpha$  receptors, but not Ret receptors.
3. The method of Claim 2, wherein said GFR $\alpha$  receptors are GFR $\alpha$ 1 receptors.
4. The method of Claim 1, wherein said nervous system cells are DRG neurons.
5. The method of Claim 4, wherein said DRG neurons are Ret (-/-).
6. The method of Claim 1, wherein said nervous system cells are neuroblastoma cells.
7. The method of Claim 1, wherein said intracellular signaling is measured as an increase in intracellular Ca<sup>2+</sup> concentration as compared to controls not incubated with said compound.
8. The method of Claim 7 further comprising determining that said test compound binds to said GPI-anchored receptors.
9. The method of Claim 1, wherein said intracellular signaling is measured as kinase activation.
10. The method of Claim 9, wherein said kinase activation is measured by (i) preparing a cell lysate, (ii) immunoprecipitating the cell lysate with an anti-

GPI-anchored receptor antibody to form an immunoprecipitate, (iii) performing an assay to measure kinase phosphorylation on said immunoprecipitate, and (iv) comparing the results with controls not incubated with said compound.

11. The method of Claim 10, wherein said antibody is anti-GFR $\alpha$ 1.
- 5 12. The method of Claim 9, wherein said kinase is a Src-type kinase.
13. The method of Claim 12, wherein activation of Src-type kinase is measured as activation of MAPK.
14. The method of claim 12, wherein activation of Src-type kinase is measured as activation of CREB.
- 10 15. The method of claim 12, wherein activation of Src-type kinase is measured as PLC $\gamma$  activation.
- 15 *Sub 2* 16. ~~A method for identifying a compound that is an antagonist of intracellular signaling effected by GPI-anchored receptors in nervous system cells comprising (i) incubating said nervous system cells having GPI-anchored receptors with a test compound in the presence of a sufficient amount of an agonist of said intracellular signaling to effect intracellular signaling, and (iii) comparing the results to controls not incubated with said compound.~~
17. The method of Claim 16, wherein said nervous system cells express GFR $\alpha$  receptors.
- 20 18. The method of Claim 17, wherein said GFR $\alpha$  receptors are GFR $\alpha$ 1 receptors.
19. The method of Claim 16, wherein said nervous system cells are DRG neurons
20. The method of Claim 19, wherein said DRG neurons are Ret (-/-).

21. The method of Claim 16, wherein said nervous system cells are neuroblastoma cells.
22. The method of Claim 16, wherein the intracellular signaling being measured is an increase intracellular  $\text{Ca}^{2+}$  concentration.
- 5 23. The method of Claim 16, wherein the intracellular signaling being measured is kinase activation.
24. The method of Claim 23, wherein said kinase activation is measured by (i) preparing a cell lysate, (ii) immunoprecipitating the cell lysate with an anti-GPI-anchored receptor antibody to form an immunoprecipitate, (iii) performing an assay to measure kinase phosphorylation on said immunoprecipitate, and (iv) comparing the results with controls not incubated with said compound.
- 10 25. The method of Claim 24, wherein said antibody is anti-GFR $\alpha$ 1.
26. The method of Claim 23, wherein said kinase is a Src-type kinase.
27. The method of Claim 26, wherein said Src-type kinase activation is measured as activation of MAPK.
- 15 28. The method of claim 26, wherein said Src-type kinase activation is measured as CREB activation.
29. The method of claim 26, wherein said Src-type kinase activation is measured as PLC $\gamma$  activation.
- 20 30. A method for identifying a compound that is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells that express GFR $\alpha$ 1 receptor, but not Ret receptor, with a test compound and (ii) determining whether intracellular signaling has been effected in said cells.
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31. The method of Claim 30, wherein said intracellular signaling is measured as an increase in intracellular  $\text{Ca}^{2+}$  concentration as compared to controls not incubated with said compound.

5 32. The method of Claim 31 further comprising determining that said test compound binds to said GPI-anchored receptors.

33. The method of Claim 30, wherein said intracellular signaling is measured as kinase activation.

10 34. The method of Claim 33, wherein said kinase activation is measured by (i) preparing a cell lysate, (ii) immunoprecipitating the detergent insoluble fraction of the cell lysate with an anti-GFR $\alpha$ 1 receptor antibody to form an immunoprecipitate, (iii) performing an assay to measure kinase phosphorylation on said immunoprecipitate, and (iv) comparing the results with controls not incubated with said compound.

35. The method of Claim 33, wherein said kinase is a Src-type kinase.

15 36. The method of Claim 33, wherein activation of Src-type kinase is measured as activation of MAPK.

37. The method of claim 33, wherein activation of Src-type kinase is measured as activation of CREB.

20 38. The method of claim 33, wherein activation of Src-type kinase is measured as PLC $\gamma$  activation.

39. A method for identifying a compound that is an antagonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells that express GFR $\alpha$ 1 receptor, but not Ret receptor, with a test compound in the presence of a sufficient amount of an agonist of said intracellular signaling

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Sub 4 > to effect intracellular signaling, and (iii) comparing the results to controls not incubated with said compound.

40. The method of Claim 39, wherein said agonist is GDNF.

5 41. The method of Claim 39, wherein said intracellular signaling is measured as an increase in intracellular  $\text{Ca}^{2+}$  concentration as compared to controls not incubated with said compound.

42. The method of Claim 39 further comprising determining that said test compound binds to said GPI-anchored receptors.

10 43. The method of Claim 39, wherein said intracellular signaling is measured as kinase activation.

15 44. The method of Claim 43, wherein said kinase activation is measured by (i) preparing a cell lysate, (ii) immunoprecipitating the detergent insoluble fraction of the cell lysate with an anti-GFR $\alpha$ 1 receptor antibody to form an immunoprecipitate, (iii) performing an assay to measure kinase phosphorylation on said immunoprecipitate, and (iv) comparing the results with controls not incubated with said compound.

45. The method of Claim 43, wherein said kinase is a Src-type kinase.

46. The method of Claim 43, wherein activation of Src-type kinase is measured as activation of MAPK.

20 47. The method of claim 43, wherein activation of Src-type kinase is measured as activation of CREB.

48. The method of claim 43, wherein activation of Src-type kinase is measured as PLC $\gamma$  activation.

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49. A method for identifying a compound which is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFR $\alpha$ 1 receptor, but not Ret receptor, with a test compound (ii) determining whether an increase in intracellular Ca<sup>2+</sup> concentration is effected in said cells as compared to controls not incubated with said compound.
50. The method of Claim 49 further comprising determining that said test compound binds to GFR $\alpha$ 1 receptors.
51. The method of Claim 49, wherein said cells are DRG Ret (-/-) neurons.
52. The method of Claim 49, wherein said cells are transformed cells.
- 10 53. The method of Claim 49, wherein said cells are neuroblastoma cells.
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54. A method for identifying a compound which is an antagonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFR $\alpha$ 1 receptors, but not Ret receptors, with a compound to be tested in the presence of a sufficient amount of an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling to cause an increase in intracellular Ca<sup>2+</sup> concentration, and (ii) determining whether a decrease in intracellular Ca<sup>2+</sup> concentration is effected, as compared with controls performed without said compound to be tested.
55. The method of Claim 54, wherein said agonist is GDNF.
- 20 56. The method of Claim 54, wherein said cells are DRG Ret (-/-) neurons.
57. The method of Claim 54, wherein said cells are transformed cells.
58. The method of Claim 54, wherein said cells are neuroblastoma cells.
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59. A method for identifying a compound which is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating

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cells which express GFR $\alpha$ 1, but not Ret, with the compound to be tested, (ii) preparing a cell lysate, (iii) immunoprecipitating the detergent insoluble fraction of the cell lysate with anti-GFR $\alpha$ 1 antibodies to form an immunoprecipitate, and (iv) performing an assay for measuring kinase phosphorylation on said immunoprecipitate.

60. The method of Claim 59, wherein said cells are DRG Ret (-/-) neurons.

61. The method of Claim 59, wherein said cells are transformed cells.

62. The method of Claim 59 wherein said cells are neuroblastoma cells.

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63. A method for identifying a compound which is an antagonist of the GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFR $\alpha$ 1, but not Ret, with the compound to be tested in the presence of a sufficient amount of an agonist of said intracellular signaling to effect kinase phosphorylation (ii) preparing a cell lysate, (iii) immunoprecipitating the detergent insoluble fraction of the cell lysate with anti-GFR $\alpha$ 1 antibodies to form an immunoprecipitate, (iv) performing an assay for measuring kinase phosphorylation on said immunoprecipitate, and (v) comparing the results of said assay to those achieved in control experiments performed in the absence of said compound to be tested.

64. The method of Claim 63 wherein said agonist is GDNF.

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65. The method of Claim 63, wherein said cells are DRG Ret (-/-) neurons.

66. The method of Claim 63, wherein said cells are transformed cells.

67. The method of Claim 63, wherein said cells are neuroblastoma cells.

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68. A method for identifying a compound which is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFR $\alpha$ 1, but not Ret, with a compound to be tested, and (ii)

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determining whether activation of Src-type kinase is effected, as compared with controls not incubated with said compound.

69. The method of Claim 68, wherein the Src-type kinase is selected from the group consisting of Fyn, c-Src, and Yes.

5 70. The method of Claim 68, wherein Src-type kinase activation is determined by measuring phosphorylation of MAPK.

71. The method of Claim 68, wherein Src-type kinase activation is determined by measuring phosphorylation of CREB.

72. The method of Claim 68, wherein said cells are DRG Ret (-/-) neurons.

10 73. The method of Claim 68, wherein said cells are transformed cells.

74. The method of Claims 68, wherein said cells are neuroblastoma cells.

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15 75. A method for identifying a compound which is an antagonist of the GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling pathway comprising (i) incubating cells which express GFR $\alpha$ 1, but not Ret, with a compound to be tested in the presence of a sufficient amount of an agonist of said pathway to cause activation of Src-type kinase and (ii) determining whether said compound effects a decrease in Src-type kinase activation, as compared with controls not incubated with said compound.

20 76. The method of Claim 75 wherein the Src-type kinase is selected from the group consisting of Fyn, c-Src and Yes.

77. The method of Claim 75, wherein Src-type kinase activation is determined by measuring phosphorylation of MAPK.

78. The method of Claim 75, wherein Src-type kinase activation is determined by measuring phosphorylation of CREB.



79. The method of Claim 75, wherein said agonist is GDNF.
80. The method of Claim 75, wherein said cells are DRG Ret (-/-) neurons.
81. The method of Claim 75, wherein said cells are transformed cells.
82. The method of Claim 75, wherein said cells are neuroblastoma cells.

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83. A method for identifying a compound which is an agonist of intracellular signaling effected by GFR $\alpha$  receptors comprising (i) incubating lipid rafts prepared from cells having GFR $\alpha$  receptors with said compound and (ii) determining whether Src-type kinase is activated as compared to controls not incubated with said compound.

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84. The method of Claim 83 wherein the Src-type kinase is selected from the group consisting of Fyn, c-Src and Yes.

85. The method of Claim 83, wherein Src-type kinase activation is determined by measuring phosphorylation of MAPK.

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86. The method of Claim 83, wherein Src-type kinase activation is determined by measuring phosphorylation of CREB.

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87. A method for identifying a compound which is an antagonist of intracellular signaling effected by GFR $\alpha$  receptors comprising (i) incubating lipid rafts prepared from cells having GFR $\alpha$  receptors with said compound in the presence of a sufficient amount of an agonist of the GFR $\alpha$ -dependent, Ret-independent intracellular signaling pathway to activate Src-type kinases and (ii) comparing the results to control experiments performed in the absence of said compound.

88. The method of Claim 87 wherein the Src-type kinase is selected from the group consisting of Fyn, c-Src and Yes.

89. The method of Claim 87, wherein Src-type kinase activation is determined by measuring phosphorylation of MAPK.
90. The method of Claim 87, wherein Src-type kinase activation is determined by measuring phosphorylation of CREB.
- 5 91. The method of Claim 87, wherein said agonist is GDNF.
92. A method for effecting a cellular response in nervous system cells comprising administering an effective amount of an agonist or antagonist of GPI-anchored intracellular signaling.
- 10 93. The method of Claim 92, wherein said nervous system cells are motor neurons.
94. The method of Claim 92, wherein said nervous system cells are sensory neurons.
95. The method of Claim 92, wherein said sensory neurons are auditory neurons.
- 15 96. The method of Claim 92, wherein said nervous system cells are glial cells.
97. The method of claims 92-97 wherein said agonist or antagonist of GPI-anchored, intracellular signaling is an agonist or antagonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.
- 20 98. A method for treating neuronal disease comprising administering an effective amount of an agent which is an agonist of GPI-anchored intracellular signaling.

99. The method of Claim 98, wherein said agonist of GPI-anchored intracellular signaling is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.

5 100. A method for treating neuronal disease comprising administering an effective amount of an agent which is an antagonist of GPI-anchored intracellular signaling.

101. The method of Claim 100, wherein said antagonist of GPI-anchored, intracellular signaling is an antagonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.

10 102. A method for treating neuronal disease comprising administering an effective amount of an agent that is an agonist of both Ret-dependent and GPI-anchored, Ret-independent intracellular signaling.

15 103. The method of Claim 102, wherein said agonist of GPI-anchored, Ret-independent intracellular signaling is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.

104. A method for treating neuronal disease comprising administering an effective amount of an agent that is an antagonist of both Ret-dependent and GPI-anchored, Ret-independent intracellular signaling.

20 105. The method of Claim 104, wherein said antagonist of GPI-anchored, Ret-independent intracellular signaling is an antagonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.

25 106. A method for treating neuronal disease comprising administering an effective amount of an agent or agents that are agonists of Ret-dependent intracellular signaling and antagonists of GPI-anchored, Ret-independent intracellular signaling.

107. The method of Claim 106, wherein said antagonists of GPI-anchored, Ret independent intracellular signaling are antagonists of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.
- 5 108. A method for treating neuronal disease comprising administering an effective amount of an agent or agents that are antagonists of Ret-dependent intracellular signaling and agonists of GPI-anchored, Ret-independent intracellular signaling.
- 10 109. The method of Claim 108, wherein said agonists of GPI-anchored, Ret independent intracellular signaling are agonists of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.
110. A method for protecting auditory neurons comprising administering an effective amount of an agent which is an agonist of GPI-anchored intracellular signaling.
- 15 111. A method for preventing memory loss comprising administering an effective amount of an agent which is an agonist of GPI-anchored intracellular signaling.
112. A method of improving learning ability comprising administering an effective amount of an agent which is an agonist of GPI-anchored intracellular signaling.
- 20 113. A method for treating epilepsy comprising administering an effective amount of an agent which is an agonist of GPI-anchored intracellular signaling.
114. A method for treating Parkinson's Disease comprising administering an effective amount of an agent which is an agonist of GPI-anchored, intracellular signaling.

115. The method of Claims 110-114 wherein said agonist of GPI-anchored, intracellular signaling is an agonist of GFR $\alpha$ 1-dependent, Ret-independent intracellular signaling.

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